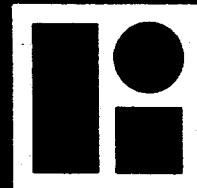


Final report-Mutagenicity Evaluation of Calcium Ascorbate F. C. C.
10/29/76

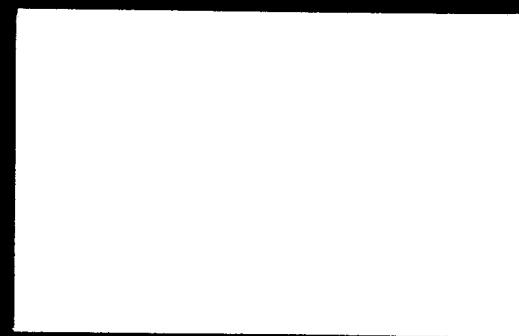
FDA 75-63

1BB



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5516 Nicholson Lane
Kensington, Maryland
20795

BRUSICK

MUTAGENICITY EVALUATION
OF
CALCIUM ASCORBATE F.C.C.
FDA 75-63
FINAL REPORT

SUBMITTED TO
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
5600 FISHERS LANE
ROCKVILLE, MARYLAND

SUBMITTED BY
LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795
LBI PROJECT NO. 2672
OCTOBER 29, 1976



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EVALUATION SUMMARY

The test compound Calcium Ascorbate F.C.C., FDA 75-63, 005743-27-1, did not exhibit mutagenic activity in any of the assays employed in these studies.



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DATE: October 29, 1976

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound Calcium Ascorbate F.C.C., FDA 75-63

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: September 3, 1976
2. Description: white crystalline powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain:	<u>Saccharomyces cerevisiae</u> , strain D4
Bacteria Strains:	<u>Salmonella typhimurium</u> , strains TA-1535
	TA-1537
	TA-1538
	TA-98
	TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	4 μ moles
2. Glucose-6-Phosphate	5 μ moles
3. Sodium Phosphate (dibasic) pH 7.4	100 μ moles
4. $MgCl_2$	8 μ moles
5. KCl	33 μ moles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	



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D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

<u>Assay</u>	<u>Chemical^a</u>	<u>Solvent</u>	<u>Probable Mutagenic Specificity</u>
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS ^b
	Ethylmethanesulfonate	Water or saline	BPS ^b
	2-Nitrofluorene	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS ^b
	2-Acetylaminofluorene	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline	Dimethylsulfoxide ^c	FS
	2-Aminoanthracene	Dimethylsulfoxide ^c	BPS ^b

^a Concentrations given in the Results Section

^b BPS = base-pair substitution; FS = frameshift

^c Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.



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B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.



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D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15 M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

2. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.



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IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: 005743-27-1

2. Test solvent: Saline

3. Solubility of the test compound under treatment conditions:
Soluble

4. Additional comments: White crystalline powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: September 8, 1976

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

<u>Test Doses</u>	<u>Percent Concentration</u>	
	Bacteria	Yeast
1/4 50% Survival	0.055	1.25
1/2 50% Survival	0.110	2.50
50% Survival	0.220	5.00



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C. Suspension Assay Results

The suspension test results for the test compound are summarized in the following six tables. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second through the fourth table of the suspension set presents the results for the activation assays. The fifth table shows the results of the nonactivation plate test and the sixth table shows the results of the activation plate test. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.



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DATA TABLE TERMS AND ABBREVIATIONS

<u>ABBREVIATION OR TERM</u>	<u>DEFINITION OR EXPLANATION</u>																														
COMPOUND	Client designated compound number appears in this column.																														
TEST CODES	<table> <tr> <td>NAN</td><td>= Nonactivation: Solvent Control</td></tr> <tr> <td>NAP</td><td>= Nonactivation: Positive Control</td></tr> <tr> <td>NA1</td><td>= Nonactivation: Test Compound Dose 1</td></tr> <tr> <td>NA2, etc.</td><td>= Reflects the other dose level(s)</td></tr> <tr> <td>A+C</td><td>= Negative Chemical Control for ACP</td></tr> <tr> <td>A-C</td><td>= Activation: Solvent Control</td></tr> <tr> <td>ALI</td><td>= Activation: Homogenate Control (Liver)</td></tr> <tr> <td>ALU</td><td>= Activation: Homogenate Control (Lung)</td></tr> <tr> <td>ACP</td><td>= Activation: Positive Control</td></tr> <tr> <td>ACT</td><td>= Activation Test</td></tr> <tr> <td>LI</td><td>= Liver Tissue Activation Fraction</td></tr> <tr> <td>LU</td><td>= Lung Tissue Activation Fraction</td></tr> <tr> <td>KI</td><td>= Kidney Tissue Activation Fraction</td></tr> <tr> <td>TE</td><td>= Testes Tissue Activation Fraction</td></tr> <tr> <td>1,2, etc.</td><td>= Dose Levels</td></tr> </table>	NAN	= Nonactivation: Solvent Control	NAP	= Nonactivation: Positive Control	NA1	= Nonactivation: Test Compound Dose 1	NA2, etc.	= Reflects the other dose level(s)	A+C	= Negative Chemical Control for ACP	A-C	= Activation: Solvent Control	ALI	= Activation: Homogenate Control (Liver)	ALU	= Activation: Homogenate Control (Lung)	ACP	= Activation: Positive Control	ACT	= Activation Test	LI	= Liver Tissue Activation Fraction	LU	= Lung Tissue Activation Fraction	KI	= Kidney Tissue Activation Fraction	TE	= Testes Tissue Activation Fraction	1,2, etc.	= Dose Levels
NAN	= Nonactivation: Solvent Control																														
NAP	= Nonactivation: Positive Control																														
NA1	= Nonactivation: Test Compound Dose 1																														
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ACT	= Activation Test																														
LI	= Liver Tissue Activation Fraction																														
LU	= Lung Tissue Activation Fraction																														
KI	= Kidney Tissue Activation Fraction																														
TE	= Testes Tissue Activation Fraction																														
1,2, etc.	= Dose Levels																														
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration																														
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = $\times 10^6$).																														
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.																														
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.																														
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.																														
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.																														
CONTAM	Presence of contamination on any plates.																														

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (<u>Macaca mulatta</u>)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES / NONACTIVATION COMPOUND 005743271

TEST	ORG	TAI100 HIS EX-8	TAI535 HIS EX-8	TAI537 HIS EX-8	TAI538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	CONTROLS
NAN		66.63	18.29	14.48	1.21	7.93	13.86	22.36	9.75
NAP		729.93	4938.27	95.62	143.90		020.41	68.71	38.10
NA1		35.86	7.26	14.35	2.99		12.66	21.45	11.73
NA2		57.53	10.75	12.12	4.62	7.74	17.51	12.88	7.54
NA3		56.33	13.10	14.34	7.03	8.64	9.00	18.08	13.62

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES ICRFL0/ MOUSE COMPOUND 005743271

TEST	ORG	TA100			TA1535			TA1537			TA1538			TA98			0000D4			0000D4				
		HIS	HIS	HIS	EX-8	EX-8	EX-8	EX-8	EX-8	EX-8	EX-8	EX-8	EX-8	EX-8	EX-8	ADE	TRY	TRY	EX-5	EX-5	EX-5			
ACT	A+C	20.58	7.58	2.69				29.04			4.72			23.27			8.14							
ACT	A-C	22.30	5.57	5.85				28.54			3.09			26.15			7.98							
ACT	AL1	24.42	6.44	6.95				49.71			9.94			26.53			8.68							
ACT	ALU	21.77	7.57	3.65				25.54			5.41			28.89			8.63							
ACT	PLI	70.14	136.26	142.11				202.67			106.30			67.69			21.36							
ACT	PLU	22.31	27.00	2.14				38.36			110.39			32.40			11.43							
ACT	L11	19.27	9.52	6.97				26.87			14.46			25.25			13.65							
ACT	L12	23.81	10.96	7.25				30.76			14.03			22.98			12.31							
ACT	L13	20.11	3.73	4.94				10.98			11.61			21.94			9.78							
ACT	L01	24.08	15.81	10.49				28.47			6.39			24.62			11.76							
ACT	L02	23.30	8.45	12.02				29.04			9.51			23.54			13.28							
ACT	L03	24.20	4.17	9.31				6.31			7.74			30.49			11.34							

LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES SPRAEW/HAI COMPOUND 005743271

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
ACT	A+C	20.72	10.78	4.17	4.92	15.89	43.00	21.56
ACT	A-C	25.83	10.16	2.65	4.12	12.44	51.25	20.37
ACT	ALI	31.45	13.54	3.74	10.45	11.62	16.09	41.81
ACT	ALU	27.04	12.64	2.11	7.49	12.41	20.06	15.18
ACT	PLI	61.73	246.86	122.50	210.59	84.05	79.89	60.49
ACT	PLU	28.22	13.97	1.37	273.20	24.85	40.92	22.52
ACT	L11	32.21	23.44	1.64	14.72	34.47	11.61	44.07
ACT	L12	25.21	14.90	1.71	17.09	34.19	10.84	38.60
ACT	L13	35.35	13.25	1.17	11.54	20.89	36.33	17.83
ACT	L01	31.00	30.93	1.82	40.00	12.08	22.70	49.30
ACT	L02	41.06	15.79	1.57	20.51	9.56	22.01	41.67
ACT	L03	30.67	9.64	1.98	12.70	18.95	49.70	25.82

NEGATIVE CONTROLS

LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES RHECUS/MONKEY COMPOUND 005743271

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	NEGATIVE CONTROLS
ACT	A+C	26.73	7.45	11.79	3.31	26.43	13.21	7.50
ACT	A-C	25.84	9.64	7.98	6.21	27.12	16.19	7.53
ACT	ALI	30.06	6.89	18.45	10.77	56.55	15.71	7.72
ACT	ALU	28.62	7.57	22.19	3.79	61.49	8.72	5.72
ACT	PLI	60.22	58.15	3.07	580.84	88.51	67.41	24.55
ACT	PLU	30.95	8.82	13.13	3.86	42.92	12.93	6.12
ACT	L11	30.68	15.53	11.25	26.36	48.29	21.33	6.23
ACT	L12	23.10	11.63	19.03	20.51	37.56	15.07	6.71
ACT	L13	27.50	10.34	23.10	24.22	37.09	18.26	7.15
ACT	L01	27.50	8.05	23.20	7.28	34.39	12.30	7.44
ACT	L02	24.61	8.27	18.11	7.62	36.74	9.07	5.22
ACT	L03	30.70	8.70	17.33	8.33	35.48	10.72	5.81

SUMMARY_OF_RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 005743271
 B. TEST DATE: OCT. 12, 1976

TEST	SPECIES	ISSUE	PLATE-IESIS		PLATE-EHRMAN		PLATE	
			IA-1535	IA-1537	IA-1538	IA-98	IA-98	IA-100
1. NON-ACTIVATION								
SOLVENT CONTROL*	RAT	---	31	23	31	19	22	21
POSITIVE CONTROL**	RAT	---	>1000	>1000	895	>1000	>1000	>1000
TEST	0.22000 %	---	36	23	11	14	24	40
	0.11000 %	---	36	20	20	11	17	287
	0.05500 %	---	20	19	19	10	22	261
							24	224
							35	241
2. ACTIVATION								
SOLVENT CONTROL*	MOUSE	LIVER	25	40	20	12	22	24
	RAT	LIVER	20	20	14	11	32	40
	MONKEY	LIVER	16	41	12	6	22	59
	MOUSE	LIVER	202	154	303	516	>1000	129
	RAT	LIVER	94	91	>1000	127	462	167
	MONKEY	LIVER	513	375	80	119	>1000	239
	MOUSE	LIVER	41	43	21	24	>1000	173
	MOUSE	LIVER	36	45	19	25	16	57
	MOUSE	LIVER	28	56	22	27	21	60
							27	51
							16	52
							35	43
							29	43
								139
								130
								151
								133
								151
								139
0.22000 %	RAT	LIVER	16	14	36	37	21	23
0.11000 %	RAT	LIVER	19	15	13	12	25	16
0.05500 %	RAT	LIVER	20	18	14	18	17	16
							16	41
							46	49
							41	76
							76	65
								65
								65
0.22000 %	MONKEY	LIVER	32	35	13	6	27	30
0.11000 %	MONKEY	LIVER	44	19	10	5	22	23
0.05500 %	MONKEY	LIVER	34	29	10	12	18	19
							84	69
							69	80
							56	95
							56	83
								105

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** IA-1535 MNNG 2 ug/PLATE
 TA-1537 DM 20 ug/PLATE
 TA-1538 NF 100 ug/PLATE
 TA-98 NF 100 ug/PLATE
 TA-100 MNNG 2 ug/PLATE
 NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

*** IA-1535 ANTH 100 ug/PLATE
 IA-1537 AMG 100 ug/PLATE
 IA-1538 AAF 100 ug/PLATE
 IA-98 AAF 100 ug/PLATE
 TA-100 ANTH 100 ug/PLATE

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound: Calcium Ascorbate F.C.C., FDA 75-63, 005743-27-1.

A. Salmonella typhimurium

1. Plate Tests

The results of these tests were negative.

2. Nonactivation Suspension Tests

NA₂ and NA₃ doses with strain TA-1538 were repeated because of increased mutant frequencies. The repeat tests were negative. All other tests were negative.

3. Activation Suspension Tests

LU₁ and LU₂ doses with TA-1538 and LI₁ and LI₂ doses with TA-98 using rat tissue were repeated because of increased mutant frequencies. Repeat tests were negative. All other tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation Suspension Tests

The tests were all negative with D4 strain.

2. Activation Suspension Tests

The tests were all negative.

C. Conclusions

The test compound Calcium Ascorbate F.C.C., FDA 75-63, 005743-27-1, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

David J. Brusick, Ph.D. Date
Director
Department of Genetics

Reviewed by:

Robert J. Weir 10/28/71

Robert J. Weir, Ph.D. Date
Vice President



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VII. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.



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D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical which produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical which produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test which cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.



Litton BIONETICS

VIII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the his^r cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.



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C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

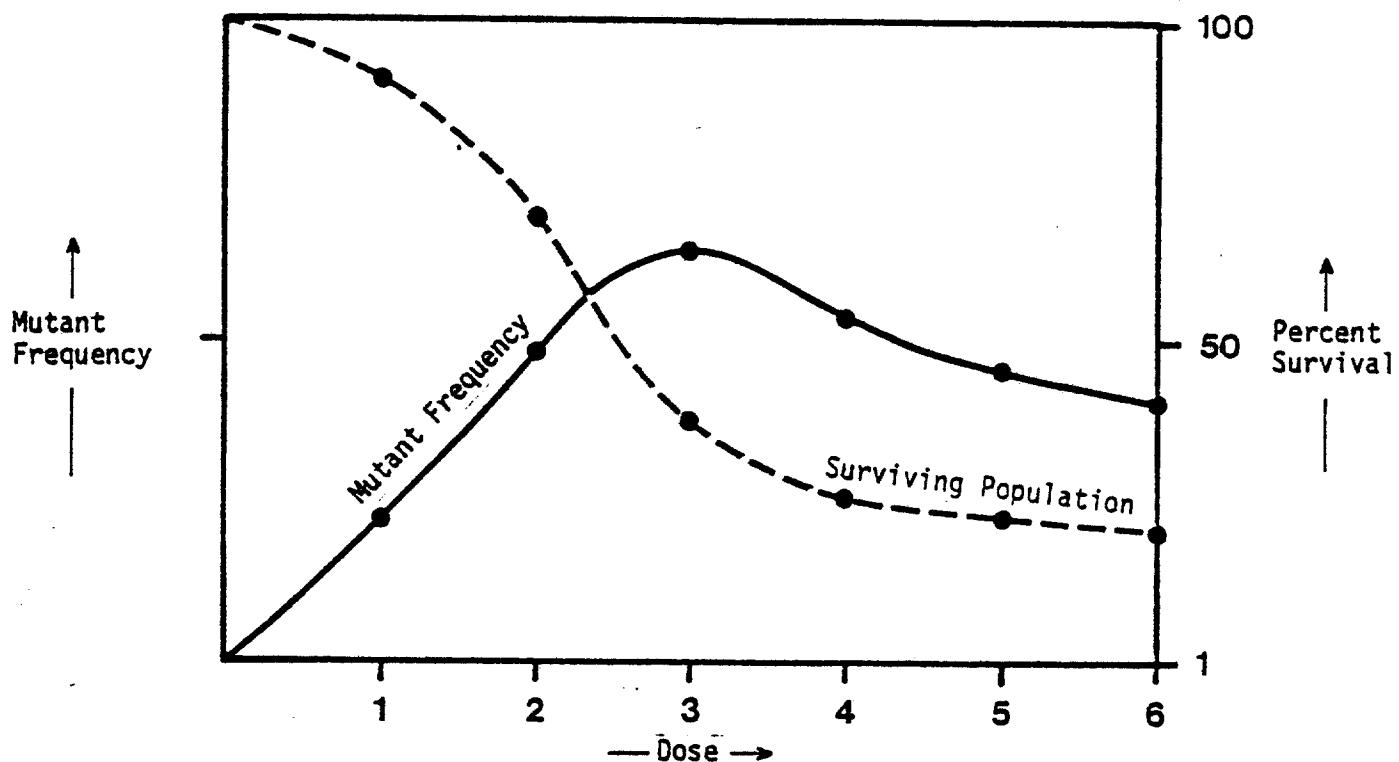
D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT

- (1) Dose levels 1, 2 & 3 were used
- (2) Dose levels 2, 3 & 4 were used
- (3) Dose levels 3, 4 & 5 were used

OBSERVED DOSE RESPONSE

- A typical positive dose response set of data would be obtained.
- The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
- Here an inverted dose response would be observed with the highest dose level showing the lowest response.

STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.



BIONETICS

APPENDIX

Tabulation of Data



LITTON BIONETICS

REPORT EXP33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	DETECTOR	TA100	SPECIES	PROJECT	DATE
627205	22374-2104			/	02468	- 10/27/76
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1
				EP+6	EP+0	EP-0
NAN	SOLVENT		0803	0535	66.63	0
NAP	EMS	0.066%	0548	4000	729.93	0
005743271	NA1	0022-2 PCT.	0976	0350	35.86	0
005743271	NA2	0011-2 PCT.	0883	0508	57.53	0
005743271	NA3	0055-3 PCT.	0829	0467	56.33	0

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST	DETECTION	SPECIES	PROJECT	DATE	
625801	22374-2104	ID	TAI535	/	02468	- 10/27/76	
COMPOUND	ORG	TEST ID	CONCENTRATION	POPU	MUT1	FREQ1	
NAN		SOLVENT	EP+6	EP+0	EP-8	EP-8	
NAP		EMS	0.2%	0081	4000	4938.27	0
005743271	NA1		0022-2 PCT.	0358	0026	7.26	0
005743271	NA2		0011-2 PCT.	0186	0020	10.75	0
005743271	NA3		0055-3 PCT.	0229	0030	13.10	0

REPORT EXR33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626501			CONTRACT 22374-2104 DETECTOR TA1537			SPECIES /			PROJECT 0246B		DATE - 10/27/76	
COMPOUND	TEST	ORG ID	CONCENTRATION			POPUL	MUT1	MUT2	FREQ1	FREQ2	CONTAM	
NAN		SOLVENT				0221	0.032		14.48		0	
NAP		QM 13 UG/ML				0251	0.240		95.62		0	
005743271	NA1		0022-2 PCI.			0230	0.033		14.35		0	
005743271	NA2		0011-2 PCI.			0297	0.036		12.12		0	
005743271	NA3		0055-3 PCI.			0265	0.038		14.34		0	

REPORT EXH33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	DETECTOR	TAI538	SPECIES	PROJECT	DATE
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1
				EP+6	EP+0	EP-0
NAN	SOLVENT		0911	0011		CONTAM
NAP	NF 667	UG/ML	0410	0590		
005743271	NA1	0022-2 PCT.	0334	0010	143.90	0
005743271	NA2	0011-2 PCT.	0411	0019	1.21	0
005743271	NA3	0055-3 PCT.	0256	0010	2.99	0
					4.62	0
					7.03	0

REPORT EXR33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST ID	DETECTOR	TA1538	SPECIES	PROJECT	DATE
628902	22374-2104					02468	- 10/27/76
COMPOUND	ORG	ID	CONCENTRATION		POPU	MUTL	FREQ1
					EP+6	EP+0	EP-B
NAN	SOLVENT			3430	0272		7.93
005743271	NA2	0011-2	PCT.	2610	0202		7.74
005743271	NA3	0055-3	PCT.	3125	0270		8.64

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 625905			CONTRACT 22374-2104 DETECTOR TA98			SPECIES /			PROJECT 02468		DATE - 10/27/76	
COMPOUND	TEST ID	CONCENTRATION	0PG	0ID	CONCENTRATION	POPU	MUTL	EP+0	FRE01	EP-0	CONTAM	
NAN	SOLVENT					0202	0028		13.86		0	
NAP	NF	667 UG/ML				0049	0402		820.41		0	
005743271	NA1	0022-2 PCT.				0403	0051		12.66		0	
005743271	NA2	0011-2 PCT.				0257	0045		17.51		0	
005743271	NA3	0055-3 PCT.				0306	0030		9.80		0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 62ABU1			CONTRACT 22374-2104	DETECTOR 0000D4	SPECIES	PROJECT 0246B			DATE - 10/27/76	
COMPOUND	TEST ID	ORG	CONCENTRATION	POPU	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM	
NAN		SOLVENT		1110	0250	0109	22.36	9.75	0	
NAP		EMS 1.0 %		0294	0202	0112	68.71	38.10	0	
005743271	NA1	0005-0 PCT.		0H44	0181	0099	21.45	11.73	0	
005743271	NA2	0025-1 PCT.		1234	0159	0093	12.00	7.54	0	
005743271	NA3	0125-2 PCT.		0896	0162	0122	18.08	13.62	0	

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627805			CONTRACT 22374-2104	DETECTOR TA100	SPECIES ICRFLO/MOUSE	PROJECT 02468	DATE - 10/27/76
COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP	MUTL	FREQ1	CONTAM
A+C		DMN 90 UM/ML	2342	0482	20.58	EP-8	0
A-C		SOLVENT	2274	0507	22.30		0
ALI		TISSUE	2604	0636	24.42		0
ALU		TISSUE	2104	0450	21.77		0
ACP	LI	DMN 90 UM/ML	1400	0982	70.14		0
ACP	LU	DMN 90 UM/ML	2506	0559	22.31		0
005743271	ACT	L11 0022-2 PCT.	3290	0634	19.27		0
005743271	ACT	L12 0011-2 PCT.	2818	0671	23.81		0
005743271	ACT	L13 0055-3 PCT.	2432	0489	20.11		0
005743271	ACT	L01 0022-2 PCT.	1902	0458	24.08		2
005743271	ACT	L02 0011-2 PCT.	1760	0410	23.30		0
005743271	ACT	L03 0055-3 PCT.	1744	0422	24.20		0

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 625904 CONTRACT 22374-2104
DETECTOR TA1535 SPECIES ICARLO/ MOUSE
PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUTI	FREQN	CONTAM
			DMN 90 UM/ML	EP+6	EP+0	EP-8	
A+C		SOLVENT	0408	0037	7.58	0	
A-C		TISSUE	0539	0030	5.57	0	
ALI		TISSUE	0357	0023	6.44	0	
ALU		TISSUE	0383	0029	7.57	0	
ACP	LI	DMN 90 UM/ML	0353	0481	136.26	0	
ACP	LU	DMN 90 UM/ML	0337	0091	27.00	0	
005743271	ACT	L11 0022-2 PCI.	0294	0028	9.52	0	
005743271	ACT	L12 0011-2 PCI.	0356	0039	10.96	0	
005743271	ACT	L13 0055-3 PCI.	1529	0057	3.73	0	
005743271	ACT	LU1 0022-2 PCI.	0234	0037	15.81	0	
005743271	ACT	LU2 0011-2 PCI.	0284	0024	8.45	0	
005743271	ACT	LU3 0055-3 PCI.	1727	0072	4.17	0	

REPORT EXR33 LITTON HIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629301 CONTRACT 22374-2104
DETECTOR TA1537 SPECIES ICRFLO/MOUSE
PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP EP+6	MUTL EP+0	FREQ1 EP-8	CONTAM
A+C		AMQ 333 UG/ML	0595	0016		2.69	0
A-C		SOLVENT	0547	0032		5.85	0
ALI		TISSUE	0518	0036	:	6.95	2
ALU		TISSUE	0548	0020		3.65	0
ACP	L1	AMQ 333 UG/ML	0311	0444		142.77	0
ACP	LU	AMQ 333 UG/ML	0608	0013		2.14	0
005743271	ACT	L11 0022-2 PCT.	0631	0044		6.97	0
005743271	ACT	L12 0011-2 PCT.	0524	0038		7.25	0
005743271	ACT	L13 0055-3 PCT.	0688	0034		4.94	0
005743271	ACT	L01 0022-2 PCT.	0715	0075		10.49	0
005743271	ACT	L02 0011-2 PCT.	0649	0078		12.02	0
005743271	ACT	L03 0055-3 PCT.	0720	0067		9.31	0

REPORT EXP33 LITTON ALIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626001 CONTRACT 22374-2104
DETECTOR FA153B SPECIES ICRFLO/ MOUSE PROJECT 0246B

COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
A+C		ANTH	67 UG/ML	0954	0277	29.04	0
A-C		SOLVENT		0862	0246	28.54	0
ALI		TISSUE		0523	0260	49.71	0
ALU		TISSUE		0924	0236	25.54	0
ACP	L1	ANTH	67 UG/ML	0449	0910	202.67	0
ACP	LU	ANTH	67 UG/ML	0842	0323	38.36	0
005743271	ACT	L11	0022-2 PCI.	0696	0187	26.87	0
005743271	ACT	L12	0011-2 PCI.	0608	0187	30.76	0
005743271	ACT	L13	0055-3 PCI.	0346	0038	10.98	0
005743271	ACT	L01	0022-2 PCI.	0713	0203	28.47	0
005743271	ACT	L02	0011-2 PCI.	0830	0241	29.04	0
005743271	ACT	L03	0055-3 PCI.	0373	0031	8.31	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627206 CONTRACT 22374-2104 PROJECT 0246H
DETECTOR 1A93 SPECIES ICRFLO/MOUSE DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUTL	FREOI	CONTAM
				EP+6	EP+0	EP-8	
A-C		ANTH 67	UG/ML	0657	0031	4.72	0
A-C		SOLVENT		1003	0031	3.09	0
ALI		TISSUE		0714	0071	9.94	0
ALU		TISSUE		0702	0038	5.41	0
ACP.	LI	ANTH 67	UG/ML	0603	0641	106.30	0
ACP	LU	ANTH 67	UG/ML	0770	0850	110.39	0
005743271	ACT	L11	0022-2 PCT.	0415	0060	14.46	0
005743271	ACT	L12	0011-2 PCT.	0449	0063	14.03	0
005743271	ACT	L13	0055-3 PCT.	0491	0057	11.61	0
005743271	ACT	L01	0022-2 PCT.	0417	0035	8.39	0
005743271	ACT	L02	0011-2 PCT.	0431	0041	9.51	0
005743271	ACT	L03	0055-3 PCT.	0530	0041	7.74	0

REPORT EXH33 LITTON BIOMEDICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629202 CONTRACT 22374-2104
SPECIES ICRFLU/ MOUSE DATE - 10/27/76

PROJECT 02468

COMPOUND	TEST ID	ORG CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
A+C	DMN 90 UM/ML	1474	0343	0120	23.27	8.14	0	
A-C	SOLVENT	1304	0341	0104	26.15	7.98	0	
ALI	TISSUE	1244	0330	0108	26.53	8.68	0	
ALU	TISSUE	1194	0345	0103	28.89	8.63	0	
ACP	LI DMN 90 UM/ML	0848	0574	0232	67.69	27.36	0	
ACP	LU DMN 90 UM/ML	1111	0360	0127	32.40	11.43	0	
005743271	ACT LI1 0005-0 PCT.	1018	0257	0139	25.25	13.65	0	
005743271	ACT LI2 0025-1 PCT.	1210	0278	0149	22.98	12.31	0	
005743271	ACT LI3 0125-2 PCT.	1258	0276	0123	21.94	9.78	0	
005743271	ACT LU1 0005-0 PCT.	1190	0293	0140	24.62	11.76	0	
005743271	ACT LU2 0025-1 PCT.	1024	0241	0136	23.54	13.28	0	
005743271	ACT LU3 0125-2 PCT.	1358	0414	0154	30.49	11.34	0	

REPORT EX-33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST ID	ORG	DETECTOR	CONCENTRATION	SPECIES	SPRDAM/RAT	PROJECT	DATE -
COMPUND						POPUP	MUT1 EP+6	FREQ1 EP-B	CONTAM
A+C			DMN	90	UM/ML	2500	0518	20.72	0
A-C			SOLVENT			2288	0591	25.83	0
ALI			TISSUE			2172	0683	31.45	0
ALU			TISSUE			2008	0543	27.04	0
ACP	LI		DMN	90	UM/ML	1419	0876	61.73	0
ACP	LU		DMN	90	UM/ML	2254	0636	26.22	0
005743271	ACT	L11	0022-2	PCT.		2164	0697	32.21	0
005743271	ACT	L12	0011-2	PCT.		2388	0602	25.21	0
005743271	ACT	L13	0055-3	PCT.		1816	0642	35.35	0
005743271	ACT	L01	0022-2	PCT.		1974	0612	31.00	0
005743271	ACT	L02	0011-2	PCT.		1572	0658	41.86	2
005743271	ACT	L03	0055-3	PCT.		2188	0671	30.67	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626701 CONTRACT 22374-2104 DETECTOR TA1535 PROJECT 02468
SPECIES SPRDAM/RAT DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
			DMN 90 UM/ML	EP+6	EP+0	EP-B	
A+C			DMN 90 UM/ML	0909	0098	10.78	0
A-C		SOLVENT		1063	0108	10.16	0
ALI		TISSUE		0672	0091	13.54	0
ALU		TISSUE		0823	0104	12.64	2
ACP	L1	DMN 90 UM/ML		1272	3140	246.86	0
ACP	LU	DMN 90 UM/ML		0687	0096	13.97	2
005743271	ACT	L11	0022-2 PCI.	0064	0015	23.44	0
005743271	ACT	L12	0011-2 PCI.	0463	0069	14.90	0
005743271	ACT	L13	0055-3 PCI.	0619	0082	13.25	0
005743271	ACT	L01	0022-2 PCI.	0097	0030	30.93	2
005743271	ACT	L02	0011-2 PCI.	0494	0078	15.79	2
005743271	ACT	L03	0055-3 PCI.	0685	0066	9.64	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629501 CONTRACT 22374-2104
DETECTOR TA1537 SPECIES SPRRAW/RAT PROJECT 02468
DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQL EP-B	CONTAM
A+C		AMQ 333 UG/ML	0600	0025	4.17	0	
A-C		SOLVENT	0641	0017	2.65	0	
ALI		TISSUE	0669	0025	3.74	1	
ALU		TISSUE	0568	0012	2.11	0	
ACP	LI	AMQ 333 UG/ML	0200	0245	122.50	1	
ACP	LU	AMQ 333 UG/ML	0582	0008	1.37	2	
005743271	ACT	L11 0022-2 PCT.	0611	0010	1.64	0	
005743271	ACT	L12 0011-2 PCT.	0645	0011	1.71	1	
005743271	ACT	L13 0055-3 PCT.	0620	0011	1.77	1	
005743271	ACT	L01 0022-2 PCT.	0658	0012	1.82	1	
005743271	ACT	LU2 0011-2 PCT.	0636	0010	1.57	0	
005743271	ACT	LU3 0055-3 PCT.	0658	0013	1.98	1	

REPORT EXP33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626505 CONTRACT 22374-2104
DETECTOR TA1538 SPECIES SPRDAW/RAT

PROJECT 02468

DATE - 10/27/76

COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP	MUTI	FREQ1	FREQ2	CONTAM
A+C		ANTH 67	UG/ML	0427	0021	4.92	0	0
A-C		SOLVENT		0486	0020	4.12	0	0
ALI		TISSUE		0271	0050	18.45	0	0
ALU		TISSUE		0374	0028	7.49	0	0
ACP	LI	ANTH 67	UG/ML	0269	0588	218.59	0	0
- ACP	LU	ANTH 67	UG/ML	0250	0683	273.20	0	0
005743271	ACT	LI1	0022-2 PCT.	0163	0024	14.72	0	0
005743271	ACT	LI2	0011-2 PCT.	0158	0027	17.09	0	0
005743271	ACT	LI3	0055-3 PCT.	0260	0030	11.54	0	0
005743271	ACT	LU1	0022-2 PCT.	0040	0016	40.00	0	0
005743271	ACT	LU2	0011-2 PCT.	0117	0024	20.51	0	0
005743271	ACT	LU3	0055-3 PCT.	0378	0048	12.70	0	0

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	DETECTOR	TA1538	SPECIES	SPRDAW/RAT	PROJECT	DATE
628901	22374-2104					02468	10/27/76
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUTL	FREQ1	CONTAM
				EP+6	EP+0	EP-B	
ALU		TISSUE		1950	0242	12.41	0
005743271	ACT	LU1	0022-2 PC1.	1946	0235	12.08	2
005743271	ACT	LU2	0011-2 PC1.	2636	0252	9.56	0

REPORT EXP33 LITTON HIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626502 CONTRACT 22374-2104			DETECTION T498			SPECIES SPREAD/RAT			PROJECT 02468		DATE - 10/27/76	
COMPOUND	TEST ID	OHC	CONCENTRATION	POPUP	MUT1	FREQ1	EP+0	EP-0	CONTAM			
A+C	ANTH 67	06/ML	1712	0272		15.89		0				
A-C	SOLVENT		2147	0267		12.44		0				
ALI	TISSUE		2557	0297		11.62		0				
ALU	TISSUE		1650	0331		20.06		0				
ACP	LI	ANTH 67	06/ML	1116	0938	84.05		0				
ACP	LU	ANTH 67	06/ML	1304	0324	24.85		0				
005743271	ACT	L11	0022-2 PCT.	0969	0334	34.47		0				
005743271	ACT	L12	0011-2 PCT.	0770	0266	34.19		0				
005743271	ACT	L13	0055-3 PCT.	1149	0240	20.89		0				
005743271	ACT	L01	0022-2 PCT.	1401	0318	22.70		0				
005743271	ACT	L02	0011-2 PCT.	1404	0309	22.01		0				
005743271	ACT	L03	0055-3 PCT.	0987	0187	18.95		0				

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	DETECTOR	TA98	SPECIES	SPRDAM/RAT	PROJECT	02468	DATE	- 10/27/76
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	EP-8	CONTAM	
005743271	ACT	L11	0022-2 PCT.	0920	0140	16.09	0		
005743271	ACT	L12	0011-2 PCT.	0922	0140	15.18	0		
				0949	0092	10.94	0		

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629502 CONTRACT 22374-2104
DETECTOR Q000D4 PROJECT 02468
SPECIES SPRDAW/RAT DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
A+C		DMN 90 UM/ML	0086	0361	0191	43.00	21.56	0	
A-C		SOLVENT	0761	0390	0155	51.25	20.37	0	
ALI		TISSUE	0708	0296	0187	41.81	26.41	0	
ALU		TISSUE	0726	0284	0153	39.12	21.07	0	
ACP	L1	DMN 90 UM/ML	0567	0453	0343	79.89	60.49	0	
ACP	LU	DMN 90 UM/ML	0826	0338	0186	40.92	22.52	0	
005743271	ACT	L11 0005-0 PCT.	0742	0327	0164	44.07	22.10	0	
005743271	ACT	L12 0025-1 PCT.	0798	0308	0172	38.60	21.55	0	
005743271	ACT	L13 0125-2 PCT.	0746	0271	0133	36.33	17.83	0	
005743271	ACT	L01 0005-0 PCT.	0645	0318	0189	49.30	29.30	0	
005743271	ACT	L02 0025-1 PCT.	0720	0300	0136	41.67	18.89	0	
005743271	ACT	L03 0125-2 PCT.	0670	0333	0173	49.70	25.82	0	

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627901 CONTRACT 22374-2104 DETECTOR TA100 SPECIES Rhesus/Monkey PROJECT 02468
DATE - 10/27/76

COMPOUND	TEST	ORG	ID	CONCENTRATION	PUPU	MUTI	FREQ1	CONTAM
				DMN 90 UM/ML	EP+6	EP+0	EP-B	
A+C				1736	0464		26.73	0
A-C				1842	0476		25.84	0
ALI				2708	0814		30.06	0
ALU				2442	0699		28.62	0
ACP	L1	DMN	90 UM/ML	1184	0713		60.22	0
ACP	LU	DMN	90 UM/ML	2430	0752		30.95	0
005743271	ACT	L11	0022-2 PCT.	2402	0737		30.68	0
005743271	ACT	L12	0011-2 PCT.	2788	0644		23.10	0
005743271	ACT	L13	0055-3 PCT.	2716	0747		27.50	0
005743271	ACT	LU1	0022-2 PCT.	2240	0616		27.50	0
005743271	ACT	LU2	0011-2 PCT.	2426	0597		24.61	0
005743271	ACT	LU3	0055-3 PCT.	2192	0673		30.70	0

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 620001 CONTRACT 22374-2104 DATE 10/27/76
PROJECT 02468 SPECIES Rhesus/Monkey

COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP	MUTL	FREQ1	FREQ2	CONTAM
A+C		DMN	90 UM/ML	2201	0164	7.45	0	
A-C		SOLVENT		2313	0223	9.64	0	
ALI		TISSUE		2757	0190	6.89	0	
ALU		TISSUE		2683	0203	7.57	0	
ACP	L1	DMN	90 UM/ML	1754	1020	58.15	0	
ACP	LU	DMN	90 UM/ML	2777	0245	8.82	0	
005743271	ACT	L11	0022-2 PCT.	1320	0205	15.53	0	
005743271	ACT	L12	0011-2 PCT.	1660	0193	11.63	0	
005743271	ACT	L13	0055-3 PCT.	1877	0194	10.34	0	
005743271	ACT	L01	0022-2 PCT.	2473	0199	8.05	0	
005743271	ACT	L02	0011-2 PCT.	2405	0199	8.27	0	
005743271	ACT	L03	0055-3 PCT.	2196	0191	8.70	0	

REPORT EXP 33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104
EXPERIMENT 629701 DATE CODE TA1537 SPECIES RHECUS/MONKEY

PROJECT 0246B
DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUTI	FREQ1	CONTAM
				EP+6	EP+0	EP-8	
A+C		AMQ 333	06/ML	2174	0327	11.79	2
A-C		SOLVENT		1818	0145	7.98	2
AL1		TISSUE		0981	0181	18.45	2
ALU		TISSUE		0996	0221	22.19	2
ACP	LI	AMQ 333	06/ML	2119	0065	3.07	2
ACP	LU	AMQ 333	06/ML	2400	0315	13.13	2
005743271	ACT	L11	0022-2 PCT.	1396	0157	11.25	0
005743271	ACT	L12	0011-2 PCT.	1361	0259	19.03	0
005743271	ACT	L13	0055-3 PCT.	1039	0240	23.10	0
005743271	ACT	L01	0022-2 PCT.	1082	0251	23.20	1
005743271	ACT	L02	0011-2 PCT.	1358	0246	18.11	0
005743271	ACT	L03	0055-3 PCT.	1696	0294	17.33	0

REPORT EXP33 LITTON BIOMEDICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627104 CONTRACT 22374-2104 DATE 10/15/78 SPECIES RHESUS/MONKEY

COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP	MUT 1	FREQ1	DATE - 10/27/76
				EP+6	EP+0	EP-8	CONTAM
A+C		ANTH 67	UG/ML	0544	0018	3.31	0
A-C		SOLVENT		0306	0019	6.21	0
ALI		TISSUE		0418	0045	10.77	0
ALU		TISSUE		0554	0021	3.79	0
ACP	L1	ANTH 67	UG/ML	0334	1940	580.84	0
ACP	LU	ANTH 67	UG/ML	0674	0026	3.86	0
005743271	ACT	L11	0022-2 PCT.	0110	0029	26.36	0
005743271	ACT	L12	0011-2 PCT.	0156	0032	20.51	0
005743271	ACT	L13	0055-3 PCT.	0128	0031	24.22	0
005743271	ACT	LU1	0022-2 PCT.	0261	0019	7.28	0
005743271	ACT	LU2	0011-2 PCT.	0210	0016	7.62	0
005743271	ACT	LU3	0055-3 PCT.	0288	0024	0.33	0

REPORT EXK33 LITTON AIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104
EXPERIMENT 627401 DETECTOR TA98

PROJECT 02468
SPECIES Rhesus/Monkey

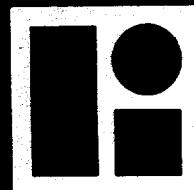
DATE - 10/27/76

COMPOUND	TEST	ORG	CONCENTRATION	POPU	MUTI	FREQ1	CONTAM
		ID		EP+6	EP+0	EP-8	
A+C		ANTH	67 UG/ML	1559	0412	26.43	1
A-C		SOLVENT		1803	0489	27.12	0
ALI		TISSUE		0916	0518	56.55	0
ALU		TISSUE		0753	0463	61.49	1
ACP	LI	ANTH	67 UG/ML	1088	0963	88.51	1
ACP	LU	ANTH	67 UG/ML	0953	0409	42.92	0
005743271	ACT	L11	0022-2 PCT.	0907	0438	48.29	0
005743271	ACT	L12	0011-2 PCT.	1001	0376	37.56	0
005743271	ACT	L13	0055-3 PCT.	1170	0434	37.09	0
005743271	ACT	L01	0022-2 PCT.	1172	0403	34.39	1
005743271	ACT	L02	0011-2 PCT.	0958	0352	36.74	1
005743271	ACT	L03	0055-3 PCT.	1133	0402	35.48	1

**REPORT EX33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPUND SUMMARY BACKUP DETAIL**

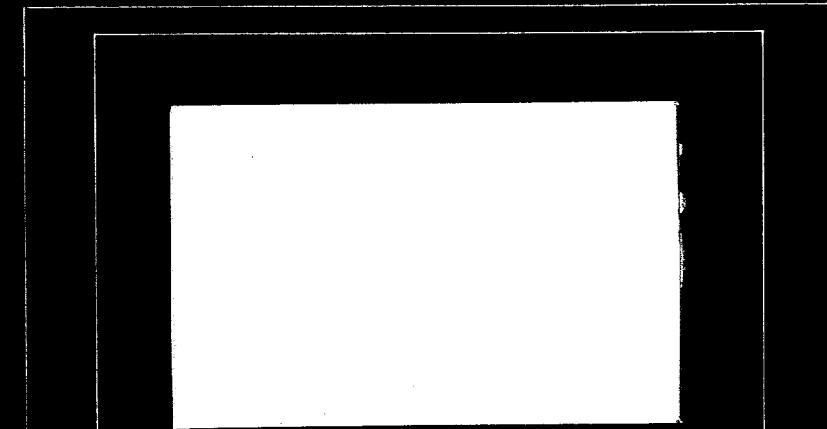
CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629503 DETECTOR 000004 SPECIES RHESUS/MONKEY DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
A+C		DMN 90 UM/ML	0787	0104	0059	13.21	7.50	0	
A-C		SOLVENT	0624	0101	0047	16.19	7.53	0	
ALI		ISSUE	0751	0110	0058	15.71	7.72	0	
ALU		ISSUE	0734	0064	0042	8.72	5.72	0	
ACP	L1	DMN 90 UM/ML	0721	0486	0177	67.41	24.55	0	
ACP	L0	DMN 90 UM/ML	0735	0095	0045	12.93	6.12	0	
005743271	ACT	L11 0005-0 PCI.	0722	0154	0045	21.33	6.23	0	
005743271	ACT	L12 0025-1 PCI.	0783	0118	0053	15.07	6.77	0	
005743271	ACT	L13 0125-2 PCI.	0657	0120	0047	18.26	7.15	0	
005743271	ACT	L01 0005-0 PCI.	0699	0086	0052	12.30	7.44	0	
005743271	ACT	L02 0025-1 PCI.	0805	0073	0042	9.07	5.22	0	
005743271	ACT	L03 0125-2 PCI.	0774	0083	0045	10.72	5.81	0	



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Kensington, Maryland
20795

MUTAGENICITY EVALUATION
OF
CALCIUM ASCORBATE F.C.C.
FDA 75-63
FINAL REPORT

SUBMITTED TO
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
5600 FISHERS LANE
ROCKVILLE, MARYLAND

SUBMITTED BY
LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795
LBI PROJECT NO. 2672
OCTOBER 29, 1976



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EVALUATION SUMMARY

The test compound Calcium Ascorbate F.C.C., FDA 75-63, 005743-27-1, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: October 29, 1976

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound Calcium Ascorbate F.C.C., FDA 75-63

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: September 3, 1976
2. Description: white crystalline powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain:	<u>Saccharomyces cerevisiae</u> , strain D4
Bacteria Strains:	<u>Salmonella typhimurium</u> , strains TA-1535
	TA-1537
	TA-1538
	TA-98
	TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	4 μ moles
2. Glucose-6-Phosphate	5 μ moles
3. Sodium Phosphate (dibasic) pH 7.4	100 μ moles
4. $MgCl_2$	8 μ moles
5. KCl	33 μ moles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	



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D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

<u>Assay</u>	<u>Chemical^a</u>	<u>Solvent</u>	<u>Probable Mutagenic Specificity</u>
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS ^b
	Ethylmethanesulfonate	Water or saline	BPS ^b
	2-Nitrofluorene	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS ^b
	2-Acetylaminofluorene	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline	Dimethylsulfoxide ^c	FS ^b
	2-Aminoanthracene	Dimethylsulfoxide ^c	BPS ^b

^a Concentrations given in the Results Section

^b BPS = base-pair substitution; FS = frameshift

^c Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose.

Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.



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B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.



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D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15 M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

2. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.



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IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: 005743-27-1
2. Test solvent: Saline
3. Solubility of the test compound under treatment conditions:
Soluble
4. Additional comments: White crystalline powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: September 8, 1976
2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

<u>Test Doses</u>	<u>Percent Concentration</u>	
	<u>Bacteria</u>	<u>Yeast</u>
1/4 50% Survival	0.055	1.25
1/2 50% Survival	0.110	2.50
50% Survival	0.220	5.00



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C. Suspension Assay Results

The suspension test results for the test compound are summarized in the following six tables. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second through the fourth table of the suspension set presents the results for the activation assays. The fifth table shows the results of the nonactivation plate test and the sixth table shows the results of the activation plate test. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.



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DATA TABLE TERMS AND ABBREVIATIONS

<u>ABBREVIATION OR TERM</u>	<u>DEFINITION OR EXPLANATION</u>																														
COMPOUND	Client designated compound number appears in this column.																														
TEST CODES	<table> <tr> <td>NAN</td><td>= Nonactivation: Solvent Control</td></tr> <tr> <td>NAP</td><td>= Nonactivation: Positive Control</td></tr> <tr> <td>NA1</td><td>= Nonactivation: Test Compound Dose 1</td></tr> <tr> <td>NA2, etc.</td><td>= Reflects the other dose level(s)</td></tr> <tr> <td>A+C</td><td>= Negative Chemical Control for ACP</td></tr> <tr> <td>A-C</td><td>= Activation: Solvent Control</td></tr> <tr> <td>ALI</td><td>= Activation: Homogenate Control (Liver)</td></tr> <tr> <td>ALU</td><td>= Activation: Homogenate Control (Lung)</td></tr> <tr> <td>ACP</td><td>= Activation: Positive Control</td></tr> <tr> <td>ACT</td><td>= Activation Test</td></tr> <tr> <td>LI</td><td>= Liver Tissue Activation Fraction</td></tr> <tr> <td>LU</td><td>= Lung Tissue Activation Fraction</td></tr> <tr> <td>KI</td><td>= Kidney Tissue Activation Fraction</td></tr> <tr> <td>TE</td><td>= Testes Tissue Activation Fraction</td></tr> <tr> <td>1,2, etc.</td><td>= Dose Levels</td></tr> </table>	NAN	= Nonactivation: Solvent Control	NAP	= Nonactivation: Positive Control	NA1	= Nonactivation: Test Compound Dose 1	NA2, etc.	= Reflects the other dose level(s)	A+C	= Negative Chemical Control for ACP	A-C	= Activation: Solvent Control	ALI	= Activation: Homogenate Control (Liver)	ALU	= Activation: Homogenate Control (Lung)	ACP	= Activation: Positive Control	ACT	= Activation Test	LI	= Liver Tissue Activation Fraction	LU	= Lung Tissue Activation Fraction	KI	= Kidney Tissue Activation Fraction	TE	= Testes Tissue Activation Fraction	1,2, etc.	= Dose Levels
NAN	= Nonactivation: Solvent Control																														
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LU	= Lung Tissue Activation Fraction																														
KI	= Kidney Tissue Activation Fraction																														
TE	= Testes Tissue Activation Fraction																														
1,2, etc.	= Dose Levels																														
CONCENTRATION	<p>All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units.</p> <p>Example: 0025-2PCT = 0.25 percent concentration</p>																														
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = $\times 10^6$).																														
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.																														
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.																														
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.																														
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.																														
CONTAM	Presence of contamination on any plates.																														

DATA TABLE TERMS AND ABBREVIATIONS (continued)

<u>ABBREVIATION OR TERM</u>	<u>DEFINITION OR EXPLANATION</u>
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (<u>Macaca mulatta</u>)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES /NONACTIVATION COMPOUND 005743271

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	CONTROLS
NAN		66.63	18.29	14.48	1.21	7.93	13.86	22.36	9.75
NAP		729.93	4938.27	95.62	143.90		820.41	68.71	38.10
NA1		35.86	7.26	14.35	2.99		12.66	21.45	11.73
NA2		57.53	10.75	12.12	4.62	7.74	17.51	12.88	7.54 TEST DATA
NA3		56.33	13.10	14.34	7.03	8.64	9.80	18.08	13.62

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES ICRFLO/MOUSE COMPOUND 005743271

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	NEGATIVE CONTROLS
ACT	A+C	20.58	7.58	2.69	29.04	4.72	23.27	8.14	
ACT	A-C	22.30	5.57	5.85	28.54	3.09	26.15	7.98	
ACT	AL1	24.42	6.44	6.95	49.71	9.94	26.53	8.68	
ACT	ALU	21.77	7.57	3.65	25.54	5.41	28.89	8.63	
ACT	PLI	70.14	136.26	142.11	202.67	106.30	67.69	21.36	POSITIVE CONTROLS
ACT	PLU	22.31	27.00	2.14	38.36	110.39	32.40	11.43	
ACT	L11	19.27	9.52	6.97	26.87	14.46	25.25	13.65	
ACT	L12	23.81	10.96	7.25	30.76	14.03	22.98	12.31	TEST DATA
ACT	L13	20.11	3.73	4.94	10.98	11.61	21.94	9.78	
ACT	L01	24.08	15.81	10.49	28.47	8.39	24.62	11.76	
ACT	L02	23.30	8.45	12.02	29.04	9.51	23.54	13.28	
ACT	L03	24.20	4.17	9.31	8.31	7.74	30.49	11.34	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES SPRUAW/RAT COMPOUND 005743271

TEST	ORG	TA100 HIS EX-B	TA1535 HIS EX-B	TA1537 HIS EX-B	TA1538 HIS EX-B	TA98 HIS EX-B	TA98 HIS EX-B	0000D4 ADE EX-5	0000D4 TRY EX-5
ACT	A+C	20.72	10.78	4.17	4.92	15.89	15.89	43.00	21.56
ACT	A-C	25.83	10.16	2.65	4.12	12.44	12.44	51.25	20.37
ACT	ALI	31.45	13.54	3.74	18.45	11.62	16.09	41.81	26.41
ACT	ALU	27.04	12.64	2.11	7.49	12.41	20.06	15.18	39.12
ACT	PLI	61.73	246.86	122.50	218.59	84.05	84.05	79.89	60.49
ACT	PLU	28.22	13.97	1.37	273.20	24.85	24.85	40.92	22.52
ACT	L11	32.21	23.44	1.64	14.72	34.47	11.61	44.07	22.10
ACT	L12	25.21	14.90	1.71	17.09	34.19	10.84	38.60	21.55
ACT	L13	35.35	13.25	1.77	11.54	20.89	20.89	36.33	17.83
ACT	L01	31.00	30.93	1.82	40.00	12.08	22.70	49.30	29.30
ACT	L02	41.86	15.79	1.57	20.51	9.56	22.01	41.67	16.89
ACT	L03	30.67	9.64	1.98	12.70	18.95	18.95	49.70	25.82

NEGATIVE CONTROLS

POSITIVE CONTROLS

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES RHESUS/MONKEY COMPOUND 005743271

TEST	ORG	TA100 HIS EX-A	TA1535 HIS EX-B	TA1537 HIS EX-B	TA1538 HIS EX-B	TA98 HIS EX-B	0000D4 ADE EX-5	0000D4 TRY EX-5	NEGATIVE CONTROLS
ACT	A+C	26.73	7.45	11.79	3.31	26.43	13.21	7.50	
ACT	A-C	25.84	9.64	7.98	6.21	27.12	16.19	7.53	
ACT	ALI	30.06	6.89	18.45	10.77	56.55	15.71	7.72	NEGATIVE CONTROLS
ACT	ALU	28.62	7.57	22.19	3.79	61.49	8.72	5.72	
ACT	PLI	60.22	58.15	3.07	580.84	88.51	67.41	24.55	POSITIVE CONTROLS
ACT	PLU	30.95	8.82	13.13	3.86	42.92	12.93	6.12	
ACT	L11	30.68	15.53	11.25	26.36	48.29	21.33	6.23	TEST DATA
ACT	L12	23.10	11.63	19.03	20.51	37.56	15.07	6.77	
ACT	L13	27.50	10.34	23.10	24.22	37.09	18.26	7.15	
ACT	L01	27.50	8.05	23.20	7.28	34.39	12.30	7.44	
ACT	L02	24.61	8.27	18.11	7.62	36.74	9.07	5.22	
ACT	L03	30.70	8.70	17.33	8.33	35.48	10.72	5.81	

SUMMARY_OF_IIESI_RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: PLATE-JESIS
B. TEST DATE: OCT. 12, 1976

TEST	B-E-V-E-B-I-A-N-I-S-P-E-R-P-L-A-T-E											
	IA-1535		IA-1537		IA-1538		IA-298		IA-29		IA-100	
SPECIES	ISSUE	1	2	1	2	1	2	1	2	1	2	
1. NON-ACTIVATION												
SOLVENT CONTROL*	---	---	---	31	23	31	19	19	18	22	21	201
POSITIVE CONTROL**	---	---	---	>1000	>1000	895	461	>1000	>1000	>1000	>1000	248
TEST	0.22000 %	---	---	36	23	11	14	26	24	43	40	>1000
	0.11000 %	---	---	36	20	20	18	11	17	40	287	266+
	0.05500 %	---	---	20	19	19	10	14	22	24	28	244
										35	241	261
												224
2. ACTIVATION												
SOLVENT CONTROL*	MOUSE	LIVER	25	40	20	12	22	23	24	19	111	123
	RAT	LIVER	20	20	14	11	32	28	40	59	89	77
	MONKEY	LIVER	16	41	12	6	22	36	51	60	57	71
	MOUSE	LIVER	202	154	303	516	>1000	>1000	167	129	123	100
	RAT	LIVER	94	91	>1000	127	462	500	239	173	154	181
	MONKEY	LIVER	513	375	80	119	>1000	>1000	142	183	167	285
	MOUSE	LIVER	41	43	21	24	16	15	52	43	139	130
	MOUSE	LIVER	36	45	19	25	21	27	16	35	133	151
	MOUSE	LIVER	28	56	22	27	19	16	35	29	141	139
	0.22000 %	RAT	16	14	36	37	21	23	73	38	57	75
	0.11000 %	RAT	19	15	13	12	25	16	46	49	72	79
	0.05500 %	RAT	20	18	14	18	17	16	41	76	65	65
	0.22000 %	MONKEY	LIVER	32	35	13	6	27	30	78	66	106
	0.11000 %	MONKEY	LIVER	44	19	10	5	22	23	84	69	80
	0.05500 %	MONKEY	LIVER	34	29	10	12	18	19	76	56	83

NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535	MNNG	2	UG/PLATE	*** TA-1535	ANTH	100	UG/PLATE
TA-1537	JM	20	UG/PLATE	TA-1537	AMQ	100	UG/PLATE
TA-1538	NF	100	UG/PLATE	TA-1538	AAF	100	UG/PLATE
TA-98	NF	100	UG/PLATE	TA-98	AAF	100	UG/PLATE
TA-100	MNNG	2	UG/PLATE	TA-100	ANTH	100	UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound: Calcium Ascorbate F.C.C., FDA 75-63, 005743-27-1.

A. Salmonella typhimurium

1. Plate Tests

The results of these tests were negative.

2. Nonactivation Suspension Tests

NA₂ and NA₃ doses with strain TA-1538 were repeated because of increased mutant frequencies. The repeat tests were negative. All other tests were negative.

3. Activation Suspension Tests

LU₁ and LU₂ doses with TA-1538 and LI₁ and LI₂ doses with TA-98 using rat tissue were repeated because of increased mutant frequencies. Repeat tests were negative. All other tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation Suspension Tests

The tests were all negative with D4 strain.

2. Activation Suspension Tests

The tests were all negative.

C. Conclusions

The test compound Calcium Ascorbate F.C.C., FDA 75-63, 005743-27-1, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

David J. Brusick, Ph.D. Date
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D. Date
Vice President
Robert J. Weir *10/28/76*



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VII. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.



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D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical which produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical which produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test which cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.



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VIII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the his⁻ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.



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C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

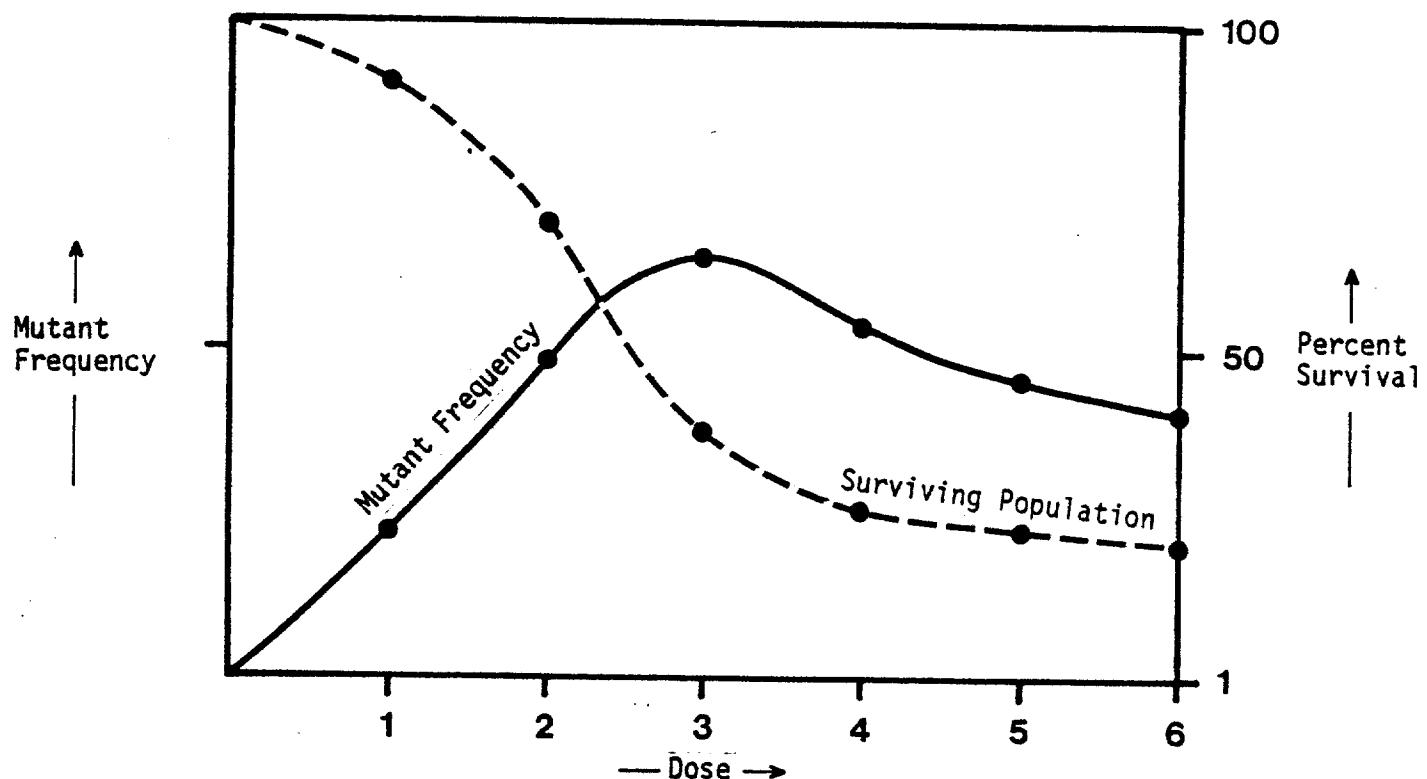
These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.



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HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT

- (1) Dose levels 1, 2 & 3 were used
- (2) Dose levels 2, 3 & 4 were used
- (3) Dose levels 3, 4 & 5 were used

OBSERVED DOSE RESPONSE

- A typical positive dose response set of data would be obtained.
- The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
- Here an inverted dose response would be observed with the highest dose level showing the lowest response.

STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.



BIONETICS

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	DETECTOR	TAC100	SPECIES	PROJECT	DATE
627205	22374-2104			/	02468	- 10/27/76
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1
NAN			SOLVENT	EP+6	EP+0	EP-B
NAP		EMS 0.066%		0548	4000	CONTAM
005743271	NA1	0022-2 PCT.		0803	0535	66.63
005743271	NA2	0011-2 PCT.		0976	0350	0
005743271	NA3	0055-3 PCT.		0883	0508	729.93
				0829	0467	0
					56.33	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 625801			CONTRACT 22374-2104	DETECTOR TA1535	SPECIES /	PROJECT 02468	DATE - 10/27/76
COMPOUND	TEST ID	ORG	CONCENTRATION	POPU	MUTL	FREQ1	CONTAM
NAN		SOLVENT	0257	0047	18.29	EP-8	0
NAP		EMS 0.2%	0081	4000	4938.27	EP-8	0
005743271	NA1	0022-2 PCT.	0358	0026	7.26	EP-8	0
005743271	NA2	0011-2 PCT.	0186	0020	10.75	EP-8	0
005743271	NA3	0055-3 PCT.	0229	0030	13.10	EP-8	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626501			CONTRACT 22374-2104	DETECTOR TA1537	SPECIES	PROJECT 02468		DATE - 10/27/76
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	FREQ2	CONTAM
NAN		SOLVENT	EP+6	EP+0		14•48	0	
NAP		QM 13 UG/ML	0251	0240		95•62	0	
005743271	NA1	0022-2 PCT.	0230	0033		14•35	0	
005743271	NA2	0011-2 PCT.	0297	0036		12•12	0	
005743271	NA3	0055-3 PCT.	0265	0038		14•34	0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	DETECTOR	TA1538	SPECIES	PROJECT	DATE
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1 EP+6	FREQ1 EP-B
NAN		SOLVENT		0911	0011	1.21
NAP		NF 667 UG/ML		0410	0590	143.90
005743271	NA1	0022-2 PCT.		0334	0010	2.99
005743271	NA2	0011-2 PCT.		0411	0019	4.62
005743271	NA3	0055-3 PCT.		0256	0018	7.03

REPORT EXR33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 628902 CONTRACT 22374-2104
PROJECT 02468 DATE - 10/21/76
DETECTOR TA1538 SPECIES /

COMPOUND	TEST ID	ORG	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
NAN	SOLVENT		EP+6	EP+0		EP-B	
005743271	NA2	0011-2	PCT.	3430	0272	7.93	0
005743271	NA3	0055-3	PCT.	2610	0202	7.74	0
				3125	0270	8.64	0

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST ID	SPECIES	PROJECT	DATE
625905	22374-2104	DETECTOR TA98	POPU	02468	- 10/27/76
COMPUND	ORG	CONCENTRATION	MUT1	FRE01	
NAN		EP+6	EP+0	EP-8	CONTAM
NAP	NF	667 UG/ML	0049	0402	13.86
005743271	NA1	0022-2 PCT.	0403	0051	020.41
005743271	NA2	0011-2 PCT.	0257	0045	12.66
005743271	NA3	0055-3 PCT.	0306	0030	17.51
					0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 62A801 CONTRACT 22374-2104 DETECTOR 0000D4 PROJECT 02468 / DATE - 10/27/76

COMPOUND	TEST ID	OKG	SPECIES	CONCENTRATION	POPU	MUT 1	MUT 2	FREQ1	FREQ2	CONTAM
					EP+4	EP+1	EP+1	EP-5	EP-5	
NAN		SOLVENT			1118	0250	0109	22.36	9.75	0
NAP		EMS 1.0 %			0294	0202	0112	68.71	38.10	0
005743271	NA1	0005-0 PCT.			0844	0181	0099	21.45	11.73	0
005743271	NA2	0025-1 PCT.			1234	0159	0093	12.88	7.54	0
005743271	NA3	0125-2 PCT.			0896	0162	0122	18.08	13.62	0

REPORT EXR33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST ID	ORG	CONCENTRATION	POP/	MUTL	FREQ1	DATE -
627805	22374-2104	DETECTOR TA100		DMN 90 UM/ML	EP+6	EP+0	EP-8	10/21/76
COMPOUND	TEST	ID						CONTAM
A+C			SOLVENT	2274	0507		20.58	0
A-C			TISSUE	2604	0636		22.30	0
ALI			TISSUE	2104	0458		24.42	0
ALU			LI	DMN 90 UM/ML	1400	0982	21.77	0
ACP			LU	DMN 90 UM/ML	2506	0559	70.14	0
ACP			LU	DMN 90 UM/ML	3290	0634	22.31	0
005743271	ACT	L11	0022-2	PCT.				
005743271	ACT	L12	0011-2	PCT.	2818	0671	19.27	0
005743271	ACT	L13	0055-3	PCT.	2432	0489	23.81	0
005743271	ACT	LU1	0022-2	PCT.	1902	0458	20.11	0
005743271	ACT	LU2	0011-2	PCT.	1760	0410	24.08	2
005743271	ACT	LU3	0055-3	PCT.	1744	0422	23.30	0
005743271	ACT						24.20	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	DETECTOR	TA1535	SPECIES	PROJECT	DATE
625904	22374-2104			ICRFL0/MOUSE	02468	- 10/27/76
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1
				EP+6	EP+0	EP-8
						CONTAM
A+C		DMN 90 UM/ML	0488	0037	7.58	0
A-C		SOLVENT	0539	0030	5.57	0
ALI		TISSUE	0357	0023	6.44	0
ALU		TISSUE	0383	0029	7.57	0
ACP	LI	DMN 90 UM/ML	0353	0481	136.26	0
ACP	LU	DMN 90 UM/ML	0337	0091	27.00	0
005743271	ACT	LJ1 0022-2 PCT.	0294	0028	9.52	0
005743271	ACT	LJ2 0011-2 PCT.	0356	0039	10.96	0
005743271	ACT	LJ3 0055-3 PCT.	1529	0057	3.73	0
005743271	ACT	LJ1 0022-2 PCT.	0234	0037	15.81	0
005743271	ACT	LJ2 0011-2 PCT.	0284	0024	8.45	0
005743271	ACT	LJ3 0055-3 PCT.	1727	0072	4.17	0

REPORT EXH33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629301		CONTRACT 22374-2104		DETECTOR FA1537		SPECIES ICRFLO/MOUSE		PROJECT 02468		DATE - 10/27/76	
COMPOUND	TEST ID	ORG	CONCENTRATION	POPUL	MUT1	POP1	MUT1	REQ1	EP-8	CONTAM	
A+C		AMQ 333 UG/ML		0595	0016			2.69		0	
A-C		SOLVENT		0547	0032			5.85		0	
ALI		TISSUE		0518	0036			6.95		2	
ALU		TISSUE		0548	0020			3.65		0	
ACP	LI	AMQ 333 UG/ML		0311	0444			142.77		0	
ACP	LU	AMQ 333 UG/ML		0608	0013			2.14		0	
005743271	ACT	LI1	0022-2 PCT.	0631	0044			6.97		0	
005743271	ACT	LI2	0011-2 PCT.	0524	0038			7.25		0	
005743271	ACT	LI3	0055-3 PCT.	0688	0034			4.94		0	
005743271	ACT	LU1	0022-2 PCT.	0715	0075			10.49		0	
005743271	ACT	LU2	0011-2 PCT.	0649	0078			12.02		0	
005743271	ACT	LU3	0055-3 PCT.	0720	0067			9.31		0	

REPORT EXP33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626001 CONTRACT 22374-2104
DETECTOR FA153B SPECIES ICRFLO/ MOUSE

PROJECT 02468

DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
				EP+6	EP+0	EP-B	
A+C		ANTH 67	UG/ML	0954	0277	29.04	0
A-C		SOLVENT		0862	0246	28.54	0
ALI		TISSUE		0523	0260	49.71	0
ALU		TISSUE		0924	0236	25.54	0
ACP	LI	ANTH 67	UG/ML	0449	0910	202.67	0
ACP	LU	ANTH 67	UG/ML	0842	0323	38.36	0
005743271	ACT	L11	0022-2 PCT.	0696	0187	26.87	0
005743271	ACT	L12	0011-2 PCT.	0608	0187	30.76	0
005743271	ACT	L13	0055-3 PCT.	0346	0038	10.98	0
005743271	ACT	L01	0022-2 PCT.	0713	0203	28.47	0
005743271	ACT	L02	0011-2 PCT.	0830	0241	29.04	0
005743271	ACT	L03	0055-3 PCT.	0373	0031	8.31	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627206 CONTRACT 22374-2104
DETECTOR 1A98 SPECIES ICRFLO/MOUSE

PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST ID	ORG	CONCENTRATION	POPU	MUT1	FRE01	CONTAM
A+C		ANTH 67	UG/ML	0657	0031	4.72	0
A-C		SOLVENT		1003	0031	3.09	0
ALI		TISSUE		0714	0071	9.94	0
ALU		TISSUE		0702	0036	5.41	0
ACP.	LI	ANTH 67	UG/ML	0603	0641	106.30	0
ACP	LU	ANTH 67	UG/ML	0770	0850	110.39	0
005743271	ACT	L11	0022-2 PCT.	0415	0060	14.46	0
005743271	ACT	L12	0011-2 PCT.	0449	0063	14.03	0
005743271	ACT	L13	0055-3 PCT.	0491	0057	11.61	0
005743271	ACT	L01	0022-2 PCT.	0417	0035	8.39	0
005743271	ACT	L02	0011-2 PCT.	0431	0041	9.51	0
005743271	ACT	L03	0055-3 PCT.	0530	0041	7.74	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629202 CONTRACT 22374-2104 DETECTOR 0000D4 SPECIES ICRFLU/ MOUSE

PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST ID	ORG CONCENTRATION	POPUP EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
A+C		DMN 90 UM/ML	1474	0343	0120	23.27	8.14	0
A-C		SOLVENT	1304	0341	0104	26.15	7.98	0
ALI		TISSUE	1244	0330	0108	26.53	8.68	0
ALU		TISSUE	1194	0345	0103	28.89	8.63	0
ACP	LI	DMN 90 UM/ML	0848	0574	0232	67.69	27.36	0
ACP	LU	DMN 90 UM/ML	1111	0360	0127	32.40	11.43.	0
005743271	ACT	L11 0005-0 PCT.	1018	0257	0139	25.25	13.65	0
005743271	ACT	L12 0025-1 PCT.	1210	0278	0149	22.98	12.31	0
005743271	ACT	L13 0125-2 PCT.	1258	0276	0123	21.94	9.78	0
005743271	ACT	L01 0005-0 PCT.	1190	0293	0140	24.62	11.76	0
005743271	ACT	L02 0025-1 PCT.	1024	0241	0136	23.54	13.28	0
005743271	ACT	L03 0125-2 PCT.	1358	0414	0154	30.49	11.34	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627801			CONTRACT 22374-2104 DETECTOR TA100		SPECIES SPHDRAW/RAT		PROJECT 02468		DATE - 10/27/76	
COMPOUND	TEST	ORG ID	CONCENTRATION		POPU	MUT1	FREQ1		CONTAM	
					EP+6	EP+0	EP-8			
A+C		DMN 90 UM/ML	2500	0518		20.72		0		
A-C	SOLVENT		2288	0591		25.83		0		
ALI	TISSUE		2172	0683		31.45		0		
ALU	TISSUE		2008	0543		27.04		0		
ACP	LI	DMN 90 UM/ML	1419	0876		61.73		0		
ACP	LU	DMN 90 UM/ML	2254	0636		28.22		0		
005743271	ACT	LI1 0022-2 PCT.	2164	0697		32.21		0		
005743271	ACT	LI2 0011-2 PCT.	2388	0602		25.21		0		
005743271	ACT	LI3 0055-3 PCT.	1816	0642		35.35		0		
005743271	ACT	LU1 0022-2 PCT.	1974	0612		31.00		0		
005743271	ACT	LU2 0011-2 PCT.	1572	0658		41.86		2		
005743271	ACT	LU3 0055-3 PCT.	2188	0671		30.67		2		

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626701 CONTRACT 22374-2104
DETECTOR TA1535 SPECIES SPRDAW/RAT

COMPOND	TEST ID	ORG	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
				EP+6	EP+0	EP-&	
A+C		DMN	90 UM/ML	0909	0098	10.78	0
A-C		SOLVENT		1063	0108	10.16	0
ALI		TISSUE		0672	0091	13.54	0
ALU		TISSUE		0823	0104	12.64	2
ACP	LI	DMN	90 UM/ML	1272	3140	246.86	0
ACP	LU	DMN	90 UM/ML	0687	0096	13.97	2
005743271	ACT	L11	0022-2 PCT.	0064	0015	23.44	0
005743271	ACT	L12	0011-2 PCT.	0463	0069	14.90	0
005743271	ACT	L13	0055-3 PCT.	0619	0082	13.25	0
005743271	ACT	L01	0022-2 PCT.	0097	0030	30.93	2
005743271	ACT	L02	0011-2 PCT.	0494	0078	15.79	2
005743271	ACT	L03	0055-3 PCT.	0685	0066	9.64	0

REPORT EXR33 LITTON AIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629501 CONTRACT 22374-2104 DETECTOR TA1537 SPECIES SPRDAM/RAT PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST ID	ORG	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
				EP+6	EP+0	EP-8	
A+C	AMQ	333 UG/ML	0600	0025	4.17	0	
A-C	SOLVENT		0641	0017	2.65	0	
ALI	TISSUE		0669	0025	3.74	1	
ALU	TISSUE		0568	0012	2.11	0	
ACP	LI	AMQ 333 UG/ML	0200	0245	122.50	1	
ACP	LU	AMQ 333 UG/ML	0582	0008	1.37	2	
005743271	ACT	L11 0022-2 PCT.	0611	0010	1.64	0	
005743271	ACT	L12 0011-2 PCT.	0645	0011	1.71	1	
005743271	ACT	L13 0055-3 PCT.	0620	0011	1.77	1	
005743271	ACT	L01 0022-2 PCT.	0658	0012	1.82	1	
005743271	ACT	LU2 0011-2 PCT.	0636	0010	1.57	0	
005743271	ACT	LU3 0055-3 PCT.	0659	0013	1.98	1	

REPORT EXR33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626505			CONTRACT 22374-2104 DETECTOR TA1538		SPECIES SPRDAW/RAT		PROJECT 02468		DATE - 10/27/76	
COMPOND	TEST	ORG ID	CONCENTRATION		POPUP	MUT1 EP+6	MUT1 EP+0	FREQ1 EP+8	CONTAM	
A+C		ANTH 67	UG/ML	0427	0021			4.92	0	
A-C		SOLVENT		0486	0020			4.12	0	
ALI		TISSUE		0271	0050			18.45	0	
ALU		TISSUE		0374	0028			7.49	0	
ACP	LI	ANTH 67	UG/ML	0269	0588			218.59	0	
ACP	LU	ANTH 67	UG/ML	0250	0683			273.20	0	
005743271	ACT	LI1	0022-2 PCT.	0163	0024			14.72	0	
005743271	ACT	LI2	0011-2 PCT.	0158	0027			17.09	0	
005743271	ACT	LI3	0055-3 PCT.	0260	0030			11.54	0	
005743271	ACT	LU1	0022-2 PCT.	0040	0016			40.00	0	
005743271	ACT	LU2	0011-2 PCT.	0117	0024			20.51	0	
005743271	ACT	LU3	0055-3 PCT.	0378	0048			12.70	0	

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 628901 CONTRACT 22374-2104 DATE - 10/27/76
DETECTOR TA1538 SPECIES SPRDAW/RAT PROJECT 02468

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
ALU		TISSUE		EP+6	EP+0	EP+8	
005743271	ACT	LU1	0022-2 PCT.	1950	0242	12.41	0
005743271	ACT	LU2	0011-2 PCT.	1946	0235	12.08	2
				2636	0252	9.56	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626502		CONTRACT 22374-2104 DETECTOR TA98		SPECIES SPRDAW/RAT		PROJECT 02468		DATE - 10/27/76	
COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP	MUT1	FREQ1	EP-8	CONTAM	
				EP+6	EP+0				
A+C	ANTH	67	UG/ML	1712	0272	15.89	0	0	
A-C	SOLVENT			2147	0267	12.44	0	0	
ALI	TISSUE			2557	0297	11.62	0	0	
ALU	TISSUE			1650	0331	20.06	0	0	
ACP	LI	ANTH	67	UG/ML	1116	0938	84.05	0	
ACP	LU	ANTH	67	UG/ML	1304	0324	24.85	0	
005743271	ACT	L11	0022-2	PCT.	0969	0334	34.47	0	
005743271	ACT	L12	0011-2	PCT.	0778	0266	34.19	0	
005743271	ACT	L13	0055-3	PCT.	1149	0240	20.89	0	
005743271	ACT	L01	0022-2	PCT.	1401	0318	22.70	0	
005743271	ACT	L02	0011-2	PCT.	1404	0309	22.01	0	
005743271	ACT	L03	0055-3	PCT.	0987	0187	18.95	0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	TEST	CONTRACT	DETECTOR	TA98	PROJECT	SPECIES	SPRUAW/RAT	DATE -
COMPOUND	ORG ID	CONCENTRATION			02468			10/27/76
ALI	TISSUE	0920	0148			MU11	FREQ1	
ALU	TISSUE	0922	0140			EP+6	EP-B	
005743271	ACT	L11	0022-2 PCT.	1361	0158		16.09	0
005743271	ACT	L12	0011-2 PCT.	0849	0092		15.18	0
							10.84	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629502 CONTRACT 22374-2104 DETECTOR 000004 SPECIES SPRDAW/RAT PROJECT 02468

DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPOP	MUT1	MUT2	FREQ1	FREQ2	CONTAM
				EP+4	EP+1	EP+1	EP-5	EP-5	
A+C		DMN 90 UM/ML	0A86	0381	0191	43.00	21.56	0	
A-C		SOLVENT	0761	0390	0155	51.25	20.37	0	
ALI		TISSUE	0708	0296	0187	41.81	26.41	0	
ALU		TISSUE	0726	0284	0153	39.12	21.07	0	
ACP	L1	DMN 90 UM/ML	0567	0453	0343	79.89	60.49	0	
ACP	LU	DMN 90 UM/ML	0826	0338	0186	40.92	22.52	0	
005743271	ACT	L11 0005-0 PCT.	0742	0327	0164	44.07	22.10	0	
005743271	ACT	L12 0025-1 PCT.	0798	0308	0172	38.60	21.55	0	
005743271	ACT	L13 0125-2 PCT.	0746	0271	0133	36.33	17.83	0	
005743271	ACT	L01 0005-0 PCT.	0645	0318	0189	49.30	29.30	0	
005743271	ACT	LU2 0025-1 PCT.	0720	0300	0136	41.67	18.89	0	
005743271	ACT	LU3 0125-2 PCT.	0670	0333	0173	49.70	25.82	0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627901 CONTRACT 22374-2104
DETECTOR TA100 SPECIES RHECUS/MONKEY
PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST	ORG	IN	CONCENTRATION	POPU	MUTL	FREQ1	CONTAM
					EP+6	EP+0	EP-8	
A+C		DMN	90	UM/ML	1736	0464	26.73	0
A-C		SOLVENT			1842	0476	25.84	0
ALI		TISSUE			2708	0814	30.06	0
ALU		TISSUE			2442	0699	28.62	0
ACP	LI	DMN	90	UM/ML	1184	0713	60.22	0
ACP	LU	DMN	90	UM/ML	2430	0752	30.95	0
005743271	ACT	L11	0022-2	PCT.	2402	0737	30.68	0
005743271	ACT	L12	0011-2	PCT.	2788	0644	23.10	0
005743271	ACT	L13	0055-3	PCT.	2716	0747	27.50	0
005743271	ACT	L01	0022-2	PCT.	2240	0616	27.50	0
005743271	ACT	LU2	0011-2	PCT.	2426	0597	24.61	0
005743271	ACT	LU3	0055-3	PCT.	2192	0673	30.70	0

REPORT EXH33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104
EXPERIMENT 628001 DETECTOR TA1535 SPECIES RHECUS/MONKEY
PROJECT 02468

DATE - 10/27/76

COMPOUND	TEST	ORG	ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
					EP+6	EP+0	EP-B	
A+C		DMN	90	UM/ML	2201	0164	7.45	0
A-C		SOLVENT			2313	0223	9.64	0
ALI		TISSUE			2757	0190	6.89	0
ALU		TISSUE			2683	0203	7.57	0
ACP	LI	DMN	90	UM/ML	1754	1020	58.15	0
ACP	LU	DMN	90	UM/ML	2777	0245	8.82	0
005743271	ACT	LI1	0005-0	PCT.	1320	0205	15.53	0
005743271	ACT	LI2	0025-1	PCT.	1660	0193	11.63	0
005743271	ACT	LI3	0125-2	PCT.	1877	0194	10.34	0
005743271	ACT	LU1	0005-0	PCT.	2473	0199	8.05	0
005743271	ACT	LU2	0025-1	PCT.	2405	0199	8.27	0
005743271	ACT	LU3	0125-2	PCT.	2196	0191	8.70	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629701			CONTRACT 22374-2104 DETECTOR TA1537		SPECIES RHECUS/MONKEY		PROJECT 02468		DATE - 10/27/76	
COMPOUND	TEST ID	ORG	CONCENTRATION	PUPU	MUT1	FREQ1			CONTAM	
				EP+6	EP+0	EP-8				
A+C		AMQ 333 UG/ML	2174	0327	11.79				2	
A-C		SOLVENT	1818	0145	7.98				2	
ALI		TISSUE	0981	0181	18.45				2	
ALU		TISSUE	0996	0221	22.19				2	
ACP	LI	AMQ 333 UG/ML	2119	0065	3.07				2	
ACP	LU	AMQ 333 UG/ML	2400	0315	13.13				2	
005743271	ACT	LI1	0022-2 PCT.	1396	0157	11.25			0	
005743271	ACT	LI2	0011-2 PCT.	1361	0259	19.03			0	
005743271	ACT	LI3	0055-3 PCT.	1039	0240	23.10			0	
005743271	ACT	LU1	0022-2 PCT.	1082	0251	23.20			1	
005743271	ACT	LU2	0011-2 PCT.	1358	0246	18.11			0	
005743271	ACT	LU3	0055-3 PCT.	1696	0294	17.33			0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627104 CONTRACT 22374-2104 DETECTOR TA1538 SPECIES RHESUS/MONKEY PROJECT 02468
DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
A+C		ANTH 67 UG/ML	0544	0018	3.31	0	
A-C	SOLVENT		0306	0019	6.21	0	
ALI	TISSUE		0418	0045	10.77	0	
ALU	TISSUE		0554	0021	3.79	0	
ACP	L1	ANTH 67 UG/ML	0334	1940	580.84	0	
ACP	LU	ANTH 67 UG/ML	0674	0026	3.86	0	
005743271	ACT	L11 0022-2 PCT.	0110	0029	26.36	0	
005743271	ACT	L12 0011-2 PCT.	0156	0032	20.51	0	
005743271	ACT	L13 0055-3 PCT.	0128	0031	24.22	0	
005743271	ACT	LU1 0022-2 PCT.	0261	0019	7.28	0	
005743271	ACT	LU2 0011-2 PCT.	0210	0016	7.62	0	
005743271	ACT	LU3 0055-3 PCT.	0288	0024	8.33	0	

REPORT EXH33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627401 CONTRACT 22374-2104 DATE - 10/27/76
DETECTOR TA98

PROJECT 02468
SPECIES RHECUS/MONKEY

COMPOUND	TEST	ORG	ID	CONCENTRATION	POPU	MUT 1	FREQ1	CONTAM
A+C		ANTH	67	UG/ML	1559	0412	26.43	1
A-C		SOLVENT			1803	0489	27.12	0
ALI		TISSUE			0916	0518	56.55	0
ALU		TISSUE			0753	0463	61.49	1
ACP	LI	ANTH	67	UG/ML	1088	0963	88.51	1
ACP	LU	ANTH	67	UG/ML	0953	0409	42.92	0
005743271	ACT	L11	0022-2	PCT.	0907	0438	48.29	0
005743271	ACT	L12	0011-2	PCT.	1001	0376	37.56	0
005743271	ACT	L13	0055-3	PCT.	1170	0434	37.09	0
005743271	ACT	L01	0022-2	PCT.	1172	0403	34.39	1
005743271	ACT	LU2	0011-2	PCT.	0958	0352	36.74	1
005743271	ACT	LU3	0055-3	PCT.	1133	0402	35.48	1

REPORT EXR33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629503 CONTRACT 22374-2104
DETECTOR 000004 SPECIES RHESUS/MONKEY

PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
A+C		DMN 90 UM/ML	0787	0104	0059	13.21	7.50	0	
A-C		SOLVENT	0624	0101	0047	16.19	7.53	0	
ALI		TISSUE	0751	0118	0058	15.71	7.72	0	
ALU		TISSUE	0734	0064	0042	8.72	5.72	0	
ACP	LI	DMN 90 UM/ML	0721	0486	0177	67.41	24.55	0	
ACP	LU	DMN 90 UM/ML	0735	0095	0045	12.93	6.12	0	
005743271	ACT	L11 0005-0 PCT.	0722	0154	0045	21.33	6.23	0	
005743271	ACT	L12 0025-1 PCT.	0783	0118	0053	15.07	6.77	0	
005743271	ACT	L13 0125-2 PCT.	0657	0120	0047	18.26	7.15	0	
005743271	ACT	L01 0005-0 PCT.	0699	0086	0052	12.30	7.44	0	
005743271	ACT	LU2 0025-1 PCT.	0805	0073	0042	9.07	5.22	0	
005743271	ACT	LU3 0125-2 PCT.	0774	0083	0045	10.72	5.81	0	